(e) *Tissue culture preparation.* Only primary cell tissue cultures shall be used in the manufacture of Measles Virus Vaccine. Continuous cell lines shall not be introduced or propagated in Measles Virus Vaccine manufacturing areas.

(f) *Control vessels.* (1) From the tissue used for the preparation of tissue cultures for growing attenuated measles virus, an amount of processed cell suspension equivalent to that used to prepare 500 ml. of tissue culture shall be used to prepare uninfected tissue control materials. This material shall be distributed in control vessels and observed microscopically for a period of no less than 14 days beyond the time of inoculation of the production vessels with measles virus; but if the production vessels are held for use in vaccine manufacture for more than 14 days, the control vessels shall be held and observed for the additional period. At the end of the observation period or at the time of virus harvest, whichever is later, fluids from the control cultures shall be tested for the presence of adventitious agents as follows:

Samples of fluid from each control vessel shall be collected at the same time as fluid is harvested from the corresponding production vessels. If multiple virus harvests are made from the same cell suspension, the control samples for each harvest shall be frozen and stored at −60° C. until the last viral harvest for that cell suspension is completed. The fluid from all the control samples from that suspension shall be pooled in proportionate amounts and at least five ml. inoculated into human and simian cell tissue culture systems and in the tissue culture system used for virus production. The cultures shall be observed for the presence of changes attributable to growth of adventitious viral agents including hemadsorption viral agents.

(2) The cell sheets of one quarter to one third of the control vessels shall be examined at the end of the observation period (14 days or longer) for the presence of hemadsorption viruses by the addition of guinea pig red blood cells. If the chick embryo cultures were not derived from a certified source (paragraph (b) of this section), the remaining tissue culture controls may be used to test for avian leucosis virus using either Rubin's procedure for detecting Resistance Inducing Factor (RIF) or a method of equivalent effectiveness.

(3) The test is satisfactory only if there is no evidence of adventitious viral agents and if at least 80 percent of the control vessels are available for observation at the end of the observation period (14 days or longer).

(g) *Test samples.* Samples of virus harvests or pools for testing by inoculation into animals, into tissue culture systems, into embryonated hens' eggs, and into bacteriological media, shall be withdrawn immediately after harvesting or pooling but prior to freezing except that samples of test materials frozen immediately after harvesting or pooling and maintained at −60° C. or below, may be tested upon thawing, provided no more than two freeze-thaw cycles are employed. The required tests shall be initiated without delay after thawing.

[38 FR 32068, Nov. 20, 1973, as amended at 40 FR 11719, Mar. 13, 1975; 47 FR 24699, June 8, 1982]

§ 630.33 Reference virus.

A U.S. Reference Measles Virus, Live, Attenuated, shall be obtained from the Center for Biologics Evaluation and Research as a control for correlation of virus titers.

[38 FR 32068, Nov. 20, 1973, as amended at 49 FR 23834, June 8, 1984; 55 FR 11013, Mar. 26, 1990]

§ 630.34 Potency test.

The concentration of live measles virus shall constitute the measure of potency. The titration shall be performed in a suitable cell culture system, free of wild viruses, using either the U.S. Reference Measles Virus, Live, Attenuated or a calibrated equivalent strain as a titration control. The concentration of live measles virus contained in the vaccine of each lot under test shall be no less than the equivalent of 1,000 $TCID_{50}$ of the U.S. reference per human dose.

§ 630.35 Test for safety.

(a) *Tests prior to clarification of vaccine manufactured in chick embryo tissue cultures.* Prior to clarification, the following tests shall be performed on each virus pool of chick embryo tissue culture:

(1) *Inoculation of adult mice.* Each of at least 20 adult mice each weighing 15–20 grams shall be inoculated intraperitoneally with 0.5 ml. and intracerebrally with 0.03 ml. amounts of each virus pool to be tested. The mice shall be observed for 21 days. Each mouse that dies after the first 24 hours of the test, or is sacrificed because of illness, shall be necropsied and examined for evidence of viral infection by direct observation and subinoculation of appropriate tissue into at least five additional mice which shall be observed for 21 days. The virus pool may be used only if at least 80 percent of the original group of mice remain healthy and survive the observation period and if none of the mice show evidence of a transmissible agent or other viral infection, other than measles virus, attributable to the vaccine.

(2) *Inoculation of suckling mice.* Each of at least 20 suckling mice less than 24 hours old shall be inoculated intracerebrally with 0.01 ml. and intraperitoneally with 0.1 ml. of the virus pool to be tested. The mice shall be observed daily for at least 14 days. Each mouse that dies after the first 24 hours of the test, or is sacrificed because of illness, shall be necropsied and examined for evidence of viral infection. Such examination shall include subinoculation of appropriate tissue suspensions into an additional group of at least five suckling mice by intracerebral and intraperitoneal routes and observed daily for 14 days. In addition, a blind passage shall be made of a single pool of the emulsified tissue (minus skin and viscera) of all mice surviving the original 14-day test. The virus pool is satisfactory for Measles Virus Vaccine only if at least 80 percent of the original inoculated mice remain healthy and survive the entire observation period, and if none of the mice used in the test show evidence of a transmissible agent or viral infection, other than measles virus, attributable to the vaccine.

(3) *Inoculation of monkey tissue cell cultures.* A volume of virus suspension of each undiluted virus pool, equivalent to at least 500 human doses or 50 milliliters, whichever represents a greater volume, shall be tested for adventitious agents in *Cercopithecus* monkey kidney tissue culture preparations or *Erythrocebus patas* monkey kidney tissue culture preparations, after neutralization of the measles virus by a high titer antiserum of nonhuman, nonsimian and nonchicken origin. The immunizing antigen used for the preparation of the measles antiserum shall be grown in tissue culture cells that shall be free of extraneous viruses which might elicit antibodies that could inhibit growth of extraneous viruses present in the measles virus pool. The tissue culture of the virus pool shall be observed for no less than 14 days. The virus pool is satisfactory for measles virus vaccine only if all the tissue culture tests fail to show evidence of any extraneous transmissible agent other than measles virus attributable to the vaccine.

(4) *Inoculation of other cell cultures.* The measles virus pool shall be tested in the same manner as prescribed in paragraph (a)(3) of this section in rhesus or cynomolgus monkey kidney, chick embryo, and human tissue cell cultures.

(5) *Inoculation of embryonated chicken eggs.* A volume of virus suspension of each undiluted virus pool, equivalent to at least 100 doses or 10 milliliters, whichever represents a greater volume, after neutralization of the measles virus by a high titer antiserum of nonhuman, nonsimian, nonavian origin shall be tested as follows:

(i) Embryonated eggs, 10 to 11 days old, shall be inoculated by the allantoic route using 0.5 milliliter per egg. Follow incubation at 35° C for 72 hours, the allantoic fluids shall be harvested, pooled, and subpassed by the same route into fresh, embryonated eggs, 10 to 11 days old, using 0.5 milliliter per egg and incubated at 35° C for 72 hours. Both the initial pool and the subpassage harvest shall be tested for the presence of hemagglutinin. The virus pool is satisfactory if the embryos appear normal and there is no evidence of hemagglutinating agents.

(ii) Embryonated eggs, 6 to 7 days old, shall be inoculated by the yolk sac route using 0.5 milliliter per egg. Following incubation at 35° C for at least 9 days, the yolk sacs shall be harvested and pooled. A 10-percent suspension of

yolk sacs shall be subpassed by the same route into fresh embryonated eggs, 6 to 7 days old, using 0.5 milliliter of inoculum per egg and incubated at 35° C for at least 9 days. The virus pool is satisfactory if the embryos in both the initial test and the subpassage appear normal.

(6) [Reserved]

(7) *Bacteriological tests.* Each virus pool shall be tested for sterility in accordance with §610.12 of this chapter. In addition each virus pool shall be tested for the presence of M. tuberculosis, both avian and human, by appropriate culture methods.

(8) *Test for avian leucosis.* If the cultures were not derived from a certified source (§630.32(b)), and the control fluids were not tested for avian leucosis (§630.32(f)), at least 500 doses or 50 ml., whichever represents a greater volume of each undiluted vaccine pool, shall be tested and found negative for avian leucosis, using either Rubin's procedure for detecting Resistance Inducing Factor (RIF) or another method of equivalent effectiveness.

(b) [Reserved]

(c) *Clarification.* After harvesting and removal of samples for testing as prescribed above in this section, the virus fluids shall be clarified by centrifugation, by passage through filters of sufficiently small porosity, or by any other method that will assure removal of all intact tissue cells which may have been collected in the harvesting process.

[38 FR 32068, Nov. 20, 1973, as amended at 40 FR 11719, Mar. 13, 1975; 41 FR 43400, Oct. 1, 1976; 47 FR 24699, June 8, 1982]

§ 630.36 General requirements.

(a) *Final container tests.* In addition to the tests required pursuant to §610.14 of this chapter, an immunological and virological identity test shall be performed on the final container if it was not performed on each pool or the bulk vaccine prior to filling.

(b)—(c) [Reserved]

(d) *Dose.* These standards are based on an individual human immunizing dose of no less than 1,000 $TCID_{50}$ of Measles Virus Vaccine Live, expressed in terms of the assigned titer of the U.S. reference measles virus.

(e) *Labeling.* In addition to the items required by other applicable labeling provisions of this subchapter, single-dose container labeling for vaccine which is not protected against photochemical deterioration shall include a statement cautioning against exposure to sunlight.

(f) [Reserved]

(g) *Photochemical deterioration; protection.* Vaccine in multiple dose final containers shall be protected against photochemical deterioration. Such containers may be colored, or outside coloring or protective covering may be used for this purpose, provided (1) the method used is shown to provide the required protection, and (2) visible examination of the contents is not precluded. Vaccine in single dose containers may be protected in the same manner provided the same conditions are met.

(h) *Sample and protocols.* The following materials shall be submitted to the Director, Center for Biologics Evaluation and Research, Food and Drug Administration, 8800 Rockville Pike, Bethesda, MD 20892:

(1) For each lot of vaccine:

(i) A protocol which consists of a summary of the history of the manufacture of the lot, including all results of each test for which test results are requested by the Director, Center for Biologics Evaluation and Research.

(ii) A total of no less than two 25-milliliter volumes in a frozen state (−60° C) of preclarification bulk vaccine containing no preservative or adjuvant.

(iii) A total of no less than 30 containers of the vaccine from each filling of each bulk lot of single-dose containers. A total of no less than six 50-dose containers or ten 10-dose containers of the vaccine from each filling of each bulk lot of multiple-dose containers.

(2) In addition to the requirements of paragraph (h)(1) of this section, whenever a new production seed lot is introduced, or whenever the source of cell culture substrate must be reestablished and recertified, samples consisting of no less than 100 milliliters in 10 milliliter volumes, in a frozen state (−60° C), of postclarification bulk vaccine